DTU Systems Biology

Course 27008 Life Science

Ch. 16: Regulation of Gene Expression

Exercises for group work

Exercises for focusing on important aspects of today's topics:

(Note: For some of the questions, you might have to go back to ch. 14 to find the answers).

Q1. List the phases involved in the lytic life cycle of a bacteriophage.

1. *Binding to a bacterial cell*
2. *Injection of phage-DNA*
3. *Replication of phage-DNA*
4. *Transcription of phage-DNA*
5. *Production of phage proteins*
6. *Host cell lysis and release of new phages*

Q2. Explain how, respectively, an inducer and a co-repressor effect expression of genes that are subjected to repressor control.

Inducers as well as co-repressors are molecules that bind repressors.
Inducer: Prevents the repressor from binding DNA -> activates transcription
Co-repressor: Promotes DNA-binding of the repressor -> limits transcription

Q3. When is it an advantage for a bacteriophage to be in the lysogenic life cycle?

If the bacterial culture is fast growing, it is an advantage for the phage to be duplicated at every cell division along with the bacteria, rather than killing the host cell as occurs in the lytic cell cycle.

Q4. A *cis*-element is defined as a DNA sequence that regulates expression of a gene placed on the same DNA molecule as the element itself. In contrast, *trans*-elements are moveable entities, usually proteins, and are able to regulate expression of genes located far away from the gene they are themselves encoded by. Use these definitions to label the below as cis- or trans-elements.

Transcription factor - *trans*

Enhancer - *cis*

Silencer - *cis*

TATA-box - *cis*

Promoter - *cis*

Repressor - *trans*

Activator – *trans*

Q5. What does each of the three eukaryotic RNA polymerases transcribe?

RNA polymerase I: rRNA
RNA polymerase II: mRNA (from protein-encoding genes, also called structural genes)
RNA polymerase III: tRNA

Q6. If one were to place a promoter from a rat gene in front of a gene from a bacterium, would the bacterium be able to transcribe the gene (explain your reasoning).

No, the prokaryotic RNA polymerase would not be able to recognize a eukaryotic promoter.

Q7. List the below processes in the order in which they take place in the cell:
_4_ 5' capping
_5_ Addition of a poly-A tail
_2_ Promoter recognition
_6_ Removal of introns
_3_ Transcription-elongation
_9_ mRNA degradation
_8_ Translation
_1_ Chromatin remodelling
_7_ mRNA transport to the cytoplasm

Q8. When protein-encoding genes are transcribed in eukaryotic cells, pre-mRNA is generated. Which three modifications of the pre-mRNA molecule are needed, before the mRNA molecule can be translated?

5' capping, addition of the poly-A tail, removal of introns.

Q9. Figure 16.17 shows a regulator protein that regulates three genes located at three different loci. Is a similar scenario possible in prokaryotes? (The regulator protein can be either an activator or a repressor).

This is also possible in prokaryotic cells. The genes must either have an operator sequence in common enabling the same repressor to bind. Alternatively they all have a site (an enhancer) for binding of the same activator.

Q10. List the different stages where protein synthesis can be regulated.

Stages (see figure 16.13):
1) *Transcription*
2) *mRNA processing (pre-mRNA splicing)*
3) *Transport of mature mRNA from the nucleus to the cytoplasm*
4) *mRNA stability*
5) *Translation*

Q11. The SRE sequence in front of some genes is also known as the 'Stress Response Element'. Is the same SRE sequence present in front of several different genes? Provide an example.

Yes, normally more genes can have the same SRE sequence, which is an example of global regulation (several genes need to be transcribed co-ordinately as a response to stress). The cells reaction to, e.g., heath or drought will result in the regulation of several genes (global regulation).

Typical exam questions (all *written* material is permitted at the exam).

Q12. (1 point).
What does the genome of an HIV particle consist of before it infects a host cell? What does the genome consist of, when HIV is present as provirus in the host cell?

Before the HIV particle infects a host cell, its genome consists of single-stranded RNA. As a provirus, the genome consists of double-stranded DNA, which is integrated into one of the host cell chromosomes.

Q13. (1 point).
Name one reason why prokaryotic cells do not need a poly-A tail on their mRNA?

The poly-A tail is responsible for mRNA export from the nucleus. Prokaryotic cells do not have a nucleus, and hence transcription and translation are not physically separated. Both processes take place in the cytoplasm.

Q14. (1 point).
A pre-mRNA molecule consists of three types of exons, A, B, and C, where A and C each are found in two variants (A1 and A2, plus C1 and C2):

After splicing, all mRNA molecules must contain an A, B, and C domain. Taking this into account, sketch the different mRNAs that can be generated by alternative splicing. What is the consequence of alternative splicing on protein level?

A1-B-C1, A1-B-C2, A2-B-C1, A2-B-C2
Alternative splicing results in different mRNA molecules and hence in different proteins.

Q15. (1 point).
What is the most important reason that the majority of human proteins cannot be synthesized in bacteria by simply cloning the genes that encode the proteins from a human chromosome, place them in front of promoters from bacteria, and insert them in a bacteria on a plasmid or a chromosome?

It is indeed possible to use cloning for placing a human gene in front of a bacterial promoter. The problem arises because human genes contain introns, and since bacteria can't perform splicing to remove the introns, the result is a non-functional protein. (Furthermore there will also be problems in the translation process).

Q16. (1 point).
What is the difference between euchromatin and heterochromatin?

Euchromatin is only lightly packed and the genes in euchromatin can be transcribed. Heterochromatin is tightly packed and the genes are normally not transcribed.

Q17. (1 point).
A gene from a eukaryotic organism is often longer than a gene from a prokaryotic organism, even though they encode proteins of equal size. Why is that?

Because eukaryotic genes often contain introns that are splices out of the mRNA prior to translation and hence do not encode amino acids.

Q18. (2 points).
Different strains of *E. coli* bacteria live in environments without glucose and without lactose. What is the level of transcription of the genes in the *lac* operon in:

A: A wild type bacteria (a bacteria with genes that are as described in the textbook)?

In surroundings without glucose, the cAMP level in the cells will be high. cAMP binds cAMP receptor protein (CRP), which enables CRP to bind the promoter of the lac *operon and thereby attract the RNA polymerase. However, when there is also no lactose in the surroundings, the lac repressor will be bound to the promoter of the* lac *operon, which prevents binding of the RNA polymerase to the promoter and thereby prevents the transcription of the genes. Accordingly, there will be no transcription from the* lac *operon.*

B: A bacteria with a nonsense mutation in the first part of the gene that encodes the lac repressor?

In a bacterium with a nonsense mutation in the first part of the gene that encodes the lac repressor, there will be no functional lac repressor. Accordingly it cannot bind the promoter of the lac *operon and it cannot prevent the binding of the RNA polymerase. The level of transcription of the genes in the* lac *operon will be high.*

Lactose is added to the growth media. What is the level of transcription of the genes in the *lac* operon in:

C: A bacteria with a nonsense mutation in the first part of the *lacZ* gene?

Explain your reasoning.

When lactose is added to the growth media, lactose will bind the lac repressor and inhibit its binding to the promoter of the lac *operon. This will enable the RNA polymerase to transcribe the genes of the* lac *operon. The level of transcription will be high and will not be affected by the nonsense mutation in the LacZ gene (it will only have a consequence at the level of translation).*

Q19. (2 point).
How will the level of degradation of lactose be in the three types of bacteria described in Q18? Explain your reasoning.

A: As described above, the transcription level of the genes of the lac operon will be zero. Accordingly, none of the proteins necessary for lactose degradation will be present and there will be no degradation.

B: As described above, the transcription level of the genes of the lac operon will be high. The proteins necessary for lactose degradation will be present, but since there is no lactose in the surroundings, no lactose degradation will take place.

C: Here, there is lactose in the surroundings and the genes of the lac operon are transcribed. However, since there is a nonsense mutation in the first part of the LacZ gene, which encodes beta-galactosidase, the enzyme responsible for lactose degradation, there will be no functional beta-galactosidase and hence no lactose degradation.